X-ray Crystallography of Concanavalin-A and IF7

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Abstract

The development of vascular (blood) supply is an essential step in the growth and metastasis of malignant tumors. Annexin 1, involved in human anti-inflammatory processes, is of use as a potential anticancer drug; it is capable of highly specific tumor vasculature recognition. Recent articles have identified the carbohydrate ligand-mimicking 7-mer peptide, IFLLWQR (IF7) as capable of targeting annexin A1 in mouse tumors. IF7 can exhibit "unprecedented tumor targeting activity", and has been detected in mouse tumors within a few minutes of intravenous injection of the peptide [PNAS, 108(49), 19587-19592 (2011)]. Thus, IF7 may have the potential to act as a delivery vehicle of anticancer drugs to the location of the tumor. Concanavalin-A is a carbohydrate binding protein, originally extracted from Jack Bean *Canavalia ensiformis*. It binds to various sugars, glycoproteins and glycolipids, by recognition of a α-D-mannosyl or a α-D-glucosyl group.

We have grown crystals of Concanavalin-A, cross-linked them with glutaraldehyde, then soaked them in a solution of IF7, in an attempt to identify the peptide's biologically active conformation.

Procedure

1. Concanavalin-A was obtained from Sigma-Aldrich (C2010). Nascently bound metals were removed by dissolving the protein in 30% (w/v) NaCl solution, then adding 1.0M HCl until ~pH 1.2. This solution was stirred at room temperature for 1 hour. Following this, it was dialyzed 3x against Milli-Q water; the pH of solution returned to ~pH 6.
2. Protein solution dialyzed 3x against 0.40M NaCl, 0.05M NaOAc, pH 5.2.
3. Protein solution centrifuged at 3220 rcf, at 4C, for 30 minutes; supernatant was filtered over 0.22um syringe filter, pellet discarded.
4. Brought protein solution to 1mM MnCl2; allowed it to incubate at room temperature for 1 hour. THEN brought solution to 1mM CaCl2.
5. Dialyzed protein solution against 0.10N NaNO3, 0.050M tris-acetate, 0.20% NaN3, 1mM MnCl2, 1mM CaCl2, pH 5.2. Concentrated protein solution to 25 mg/ml, placed solution in dialysis cassettes, allowed solution to sit quietly at room temperature for 1 week.
6. Dialyzed some cassettes against various sugar-containing solutions.
7. Cross-linked other cassettes using glutaraldehyde; placed crystals in solution containing peptide and 25% DMSO and soaked overnight.
8. Transferred crystals to mother liquor + 30% MPD; froze crystals in liquid nitrogen.
9. Examined samples using APS beamlines 19-BM and 19-ID.

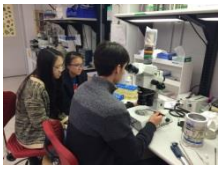

Fig 1. Freezing crystals in wet-lab.

Fig 2. Concanavalin-A crystal at 19-BM.

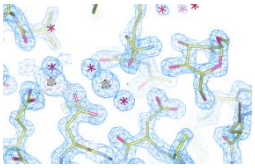

Fig 3. Ligand α D-mannopyranoside bound to active site of concanavalin-A.

Fig. 4 Collecting data at 19-BM; solving structure with bound ligand.

Results

As it turned out, an older dialysis cassette was discovered to contain a HUGE crystal of concanalin-A, projecting from the plastic of the cassette. This cassette was dialyzed for 24 hours against mother liquor containing 1% MPD and 2.5 mM methyl α D-mannopyranoside (the native ligand of concanavalin-A). The crystal was retrieved, frozen in liquid nitrogen, then analyzed on beamline 19-ID.

Other cassettes of concanavalin-A crystals were cross-linked via exposure to glutaraldehyde, then soaked in IF7 peptide/DMSO solution for 24 hours. Unfortunately, none of these crystals survived the procedure. A similar study, using a 6-mer peptide, had soaked the crystals for 20 days; this experiment could be repeated, using a longer soak time, though co-crystallization may be a better way to attempt binding the peptide in future.

Diffraction data sets were collected at a wavelength of 0.97911 A. The data allowed us to determine, *ab initio*, the phasing of the concanavalin-A protein (using the bound manganese and calcium cations). The binding sites of both cations were verified; the sugar binding site was also located and refined.

Conclusion

In this experiment we found we were working with an unusual crystal form of the concanavalin-A; space group C 2 2 21, with unit cell parameters a=101.078, b=118.300, c=250.657, α = β = γ = 90 degrees. This crystal form has only been reported once before (Foroughi, et al.), and in that study the crystals diffracted to 2.09A resolution. Our crystal, having grown very slowly, and very large, over the period of a year, diffracted to 1.45A – and showed, from an ab initio map, the binding of native ligand, a-D-mannopyranoside. This crystal form contains four monomers in the asymmetric unit; present refinement parameters are R(working) = 0.1562, R(free) = 0.1716; the final structure will be submitted to the RCSB PDB, bearing the names of all Maplewood Richmond Heights collaborators. The Advanced Photon Source's source of x-rays was invaluable to the determination of this structure.

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Acknowledgments

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